EFFECT OF INJECTION OF ACTIVE DEPOSIT OF RADIUM EMANATION ON RABBITS

WITH SPECIAL REFERENCE TO THE LEUKOCYTES AND ANTIBODY FORMATION

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Previous studies [1] on the effects of benzene, roentgen ray and thorium X on antibody formation led to an interest in the effect of radium in this respect. Radium salts in soluble form when injected in practicable doses were found to be eliminated too rapidly to produce any effect. On account of the ease of injection of the "active deposit" of radium emanation as used by Bagg [2] this form of radium seemed feasible for our work.

Bagg [3] studied the changes produced by the intravenous and subcutaneous injection in the white rat of solutions of an active deposit of radium emanation and Theis and Bagg [4] the effect of the intravenous injection in dogs. Doses from 2.6 to 10.6 millicuries given intravenously to rats did not cause death of the rats within a month. An increase from 10.6 to 11.2 millicuries was fatal in 2.5 days and all doses above this caused immediate acute effects. Subcutaneously, doses up to 10 millicuries were not fatal, while 17 millicuries killed in 5 days. After injection of active deposit of radium emanation the radioactive substance diffuses throughout the body and causes changes in the liver, lungs, kidneys, suprarenals, spleen, marrow, brain and vascular system. The liver, even after small doses subcutaneously, presents a fatty change with many giant cells and hyperchromatic nuclei which persist for a comparatively long time. After large doses, congestion and hemorrhages are frequent in practically all organs, and the animals may die with symptoms of enteritis. The most frequent change in the kidney is a granular degeneration and erosion of the tubular cells. Destruction of the cells of the marrow occurs, and in the spleen there may be found congestion with hemorrhages and destruction of red corpuscles. Intravenous injections affect the lungs more severely than the subcutaneous, producing proliferation and desquamation of the epithelial cells of the bronchi, marked edema, congestion and hemorrhage. The reaction in the tissues from radium injections is like that from radium applied externally.

Dogs tolerated as high as 146.4 millicuries in a single injection or a total of 338.4 millicuries in four intravenous injections. Large injections produce a

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[1] Hektoen: Jour. Infect. Dis., 1916, 19, p. 69; 1915, 17, p. 415; 1918, 22, p. 28; 1920, 27, p. 23. Corper: ibid., 1920, 26, p. 330.

[2] Amer. Jour. Anat., 1922, 30, p. 133.

[3] Jour. Cancer Research, 1920, 5, pp. 1 and 301.

[4] Jour. Biol. Chem., 1920, 41, p. 525.

considerable reduction in the number of leukocytes, sometimes as much as 80% of the total number, but the simultaneous reduction in red corpuscles is less, about 25%. The principal organs showed considerable congestion; the liver a general fatty and granular change, the kidneys a granular degeneration of the tubular cells, and the spleen considerable congestion, while the splenic pulp was largely drained of cells. In the marrow lymphoid tissue was replaced largely by fat.

Our experiments concern mainly the effect of intravenous injections of active deposit of radium and emanation on antibody formation, but since the toxic and other effects of radium on rabbits do not seem to have been studied, we made observations also on the changes produced in the organs and the leukocytes. Solutions of sodium chloride containing active deposit from radium emanation were used as described in detail by Bagg.[3] The radium which served as the source of the deposit is the property of the Medical and Surgical Group of Denver and amounted to 496 mg., contained in a small glass flask in the form of a slightly acid solution of the bromide salt of the element. The emanation apparatus, the property of the Radium Company of Colorado, is a modified Debierne-Duane apparatus designed by C. F. Whittemore, chief physicist of the company, to meet the Denver altitude requirements and differing in details and durability from the apparatus used by Duane[5] at sea level. To remove the emanation from the solution and purify the gas, the entire apparatus was evacuated by an electrically operated oil-sealed, rotary vacuum pump, the mercury being so manipulated that the radium emanation, together with the hydrogen and oxygen, formed through decomposition of the water by the radium radiation within the flask, diffuses into a bulb whose volume is relatively greater than that of the flask containing the radium. These mixed gases are introduced into a series of chemical chambers in which the hydrogen and oxygen come in contact with an electrically heated, slightly oxidized copper wire and form water which is absorbed in the chamber containing phosphorus pentoxide. A second mercury pump, similar to the one used in drawing the gas from the radium flask, is used to collect the purified radium emanation from the chemical chambers and transfer it into a tube in which a few milligrams of pure sodium chloride has been fused. The purified radium emanation remains in contact with the fused sodium chloride for about 3 to 4 hours, when the maximum equilibrium amount of the active deposit of rapid change has been formed. This active

[5] Bost. Med. & Surg. Jour., 1917, 177, p. 787.

deposit consists of radium A, radium B and radium C, and since radium C is the chief source of gamma radiation in any radium compound, this active deposit may be used for therapeutic purposes as long as the life of the active deposit permits. The life of this material is short, however, the gamma radiation from radium C decaying to one-half value in 60 minutes and to practically nothing at the end of 240 minutes. After the 3 to 4-hour period has elapsed, the radium emanation is drawn back into the purification apparatus and the bulb containing the active deposit on the fused sodium chloride is cut off and measured by the gamma radiation method. We usually obtained a measurement of from 120 to 230 millicuries. These measurements are made quickly to prevent loss by decay, and sterile distilled water added to dissolve the fused sodium chloride, the resulting solution containing a large portion of the radium active deposit (radium A, B and C). This is drawn into a syringe, which is then measured when containing the solution and after it has been emptied by comparison with the gamma radiation from a standard radium preparation; we obtained from 45 to 120 millicuries on the different days of preparation, which usually was 2 to 3 times a week. The instrument used was the usual gamma ray electroscope. The difference in the readings of the syringe plus solution and the empty syringe gives the approximate content of the solution that has been injected. The number of millicuries injected cannot be controlled exactly as an irregular quantity, from 20 to 50%, may remain in the syringe. The rapid decay of the active deposit introduces a second source of error. Therefore, the exact amount given can only be accurately estimated after all readings have been made.

In order to study the leukotoxic action and the changes produced by the active deposit in rabbits, graded intravenous injections, as nearly as these could be controlled, were given to a series of 13 rabbits ranging in weight from 2 to 3½ kilos. Total and differential counts were made of leukocytes before and at regular intervals after injection of the active deposit, and if the animal died the tissues were studied carefully. The results indicate that active deposit of radium emanation given intravenously in salt solution is lethal to rabbits in about 6 days in amounts exceeding approximately 20 millicuries measured by the gamma ray electroscope; an occasional animal may die from a smaller dose (16 millicuries), and larger amounts (48 millicuries) may prove fatal earlier. This form of radium produces first an increase in the circulating leukocytes as high as 34,600—as it did in a rabbit that died 4 days after the injection of 37 millicuries—which subsequently may

drop as low as 900, as it did in rabbit 3 just before death. In rabbits given a nonlethal dose the leukocytes may drop and gradually increase again, and with small amounts the counts are variable. The Arneth classification shows a shift to left with the onset of leukocytosis and then to the right as the leukocytes decrease and back to the left as a low count is reached. The mononuclear cells increase as the leukocytes fall, and in extreme cases there may be a marked relative lymphocytosis.

ANATOMIC CHANGES PRODUCED BY ACTIVE RADIUM DEPOSIT

Rabbit 1 (2.5 kilos).—Died 2 days after the intravenous injection of 48 millicuries. All veins of the skin and abdomen distended; abdominal muscles bright red; liver congested and friable, lobules well defined; spleen soft and blue; suprarenals large; kidneys pale and swollen; lungs congested, more in upper lobes and anterior; marrow of femur moist and pale. Microscopically the liver was intensely congested, the nuclei of the cells in many places pycnotic or disintegrated; the lymphoid tissue in the spleen appeared to be reduced and there was much cellular disintegration and many pigment containing cells in the splenic tissue, which was congested; the capillaries of the kidneys were dilated and there was blood in some of the collecting tubules; the epithelium of the tubules was granular, the nuclei pycnotic; the cells of the suprarenals were granular, the nuclei hyperchromatic and the vessels congested; there apparently was a decrease in the lymphoid cells in the mesenteric nodes; there were a few hemorrhages in the myocardium; the lungs were intensely congested and many of the alveoli contained blood and cellular detritus; the marrow of the femur was congested and the fatty tissues seemed to be increased, the cells diminished.

Rabbit 2 (3.1 kilos).—Died 3 days after the intravenous injection of 58 millicuries active deposit. The liver was pale and friable; spleen soft, small and purplish; kidneys pale, glomeruli congested; mesenteric lymph nodes swollen; lungs contained hemorrhagic foci, the left lung being solid anteriorly; marrow of femur pale and moist. Microscopically the liver was congested; the spleen congested, the lymphoid tissue reduced; the suprarenals congested, especially in the medulla; in the kidneys there was capillary congestion and blood in some of the tubules and glomerular capsules; there were many pigment containing cells in the mesenteric lymphoid nodes; the lungs were congested and there was blood in many bronchi and alveoli; in the marrow of the femur the cells were diminished and the fat tissue apparently increased.

Rabbit 3 (2.3 kilos).—Died 4 days after the intravenous injection of 37 millicuries active deposit. The spleen was purple and soft and the mesenteric nodes moist, otherwise the organs seemed to be normal. Microscopically there was congestion in most of the organs, the nuclei in the liver and suprarenal epithelium staining deeply; in the mesenteric lymphnodes and the marrow, the cells were reduced in number.

Rabbit 4 (2.3 kilos).—Died 5 days after the intravenous injection of 16 millicuries. The marrow of the femur was pale and much softer than normal; there was congestion in the liver, spleen, suprarenals, kidneys and lungs; in the spleen, mesenteric lymphnodes and marrow the cells seemed to be diminished in number and showed more or less disintegrative changes.

Rabbit 5 (2.5 kilos).—Died 6 days after the intravenous injection of 44 millicuries. The liver was congested and fatty; the spleen and mesenteric lymphnodes large and congested; the kidneys were also congested with a few

TABLE 1

THE LEUKOTOXIC ACTION OF INTRAVENOUS INJECTIONS OF ACTIVE DEPOSIT OF RADIUM EMANATION IN RABBITS

Amount of "Active Deposit" Given Intravenously	Rabbit	Time of Blood Examination in Relation to "Active Deposit" Injection. Days Before = B; After = A	Leukocytes on the Basis of 100 Cells Counted									
			Total per C.Mm.	Polymorphonuclears (Arneth Scale)					Eosinophils	Basophils	Mononuclears	
				I	II	III	IV	V			Large	Small
37 millicuries; fatal in 4 days	3	7 B	12,800	12	13	11	3	0	0	0	6	55
		1 B	16,700	8	13	10	1	0	0	0	4	64
	2.3 kilos	Same day	11,600	18	9	15	3	0	0	0	2	53
		1 A	34,600	52	33	10	0	0	0	0	1	4
		2 A	13,800	45	31	17	2	1	0	0	0	4
		3 A	1,000	12	13	4	0	0	0	0	7	64
		4 A	900*	..	..	..	..	..	..	...	1	17
44 millicuries; fatal in 6 days	5	1 B	11,000	29	10	10	1	0	0	0	10	40
		Same day	30,000	20	24	10	1	0	0	0	6	39
	2.5 kilos	1 A	15,000	34	33	23	4	0	0	0	1	5
		2 A	8,300	14	18	15	2	1	0	0	2	48
		3 A	4,000	12	6	3	1	0	0	0	11	67
		4 A	3,000	6	7	2	2	0	0	0	19	66
		5 A	2,700	11	4	1	0	0	0	0	16	68
		6 A	2,800	6	15	5	0	0	0	0	7	67
14.5 millicuries; not fatal	8	4 B	16,700	29	17	17	6	0	0	0	6	25
		1 B	15,500	12	20	20	4	2	0	0	9	33
	3 kilos	1 A	15,700	18	31	17	5	0	0	0	8	21
		2 A	5,000	8	23	21	11	3	0	0	4	30
		3 A	3.000	8	17	13	4	0	0	0	14	44
		4 A	2,900	10	17	17	3	0	0	0	9	47
		8 A	5,000	15	17	14	5	0	0	0	10	39
		10 A	5,000	7	13	18	1	1	0	0	12	48
		12 A	5,000	5	11	8	3	0	0	1	15	57
		14 A	6,000	13	11	8	2	0	0	0	16	50
		16 A	6,000	17	23	6	1	0	0	0	9	44
		18 A	16,000	11	11	8	1	0	0	0	12	57
		20 A	13,000	11	15	7	0	0	0	1	20	46
20 millicuries; not fatal	11	3 B	21,400	26	9	3	0	0	0	0	8	54
		Same day	25.000	21	15	5	0	0	0	0	7	52
	2.7 kilos	1 A	19,000	32	29	8	2	0	0	0	13	16
		2 A	8,400	9	18	7	2	0	0	1	3	60
		3 A	3,000	7	5	3	1	0	0	0	12	72
		4 A	2,400	6	3	4	0	0	0	1	8	78
		5 A	3,000	21	6	0	0	0	0	0	5	68
		6 A	5,200	32	6	0	0	0	0	0	0	62
		7 A	3,600	26	7	0	0	0	2	4	5	52
		9 A	4,500	21	14	0	0	0	1	0	10	54
		11 A	11,000	27	6	2	0	0	0	1	4	60

* Too few cells.

Four typical examples only are given for purpose of illustration.

TABLE 2

LYSIN AND PRECIPITIN FORMATION BY RABBITS GIVEN INTRAVENOUS INJECTIONS OF ACTIVE DEPOSIT OF RADIUM EMANATION

Treatment		Rabbits		Titers and Total Leukocytes in Relation to Antigen Injection																			
Time	Dose of Active Deposit in Milli-curies	Number	Weight in Kilos	Days Before Intraperitoneal Injection of Sheep Blood			Days After Intraperitoneal Injection of Sheep Blood																
				4	1	0	1	3	5	7	9	11	13	15	17	19	22	25	28	31	34	37	40
4 days before injection of sheep blood	10	1	4	(10.2)	(1.8)	24	0 (8.6)	0 (8.0)	192 (10.4)	1536 (5.4) 0	1536 (4.6) 3200	1536 (12.0) 3200	768 (8.2) 3200	192 (12.0) 1600	384 (9.8) 1600	768 (7.8) 1600	.... (10.8)	768 (9.6) 800	1536 (10.6) 800	1536 (10.2) 800	1536 (9.6) 800	1536 (15.0) 0	1536 (16.4) 0
4 days before injection of sheep blood	5	5	3.5	(13.0)	(54.0)	0	0 (4.0)	24 (17.0)	384 (8.8)	1536 (7.2) 500	1536 (10.6) 800	3072 (14.0) 12800	1536 (20.4) 3200	768 (17.0) 1600	768 (21.0) 1600	768 (19.0) 1600	.... (16.2)	768 (19.0) 1600	1536 (23.0) 1600	768 (23.6) 1600	768 (23.0) 800	1536 (18.0) —	768 (13.0) 0
4 days before injection of sheep blood	1	7	2.5	(15.2)	(8.0)	24	0 (10.0)	48 (14.0)	24 (16.4)	1536 (23.0) 0	3072 (12.4) 100	3072 (14.4) 800	1536 (11.2) 1600	1536 (23.0) 800	1536 (12.0) 400	1536 (12.2) 200	.... (5.8)	1536 (10.0) 200	1536 (13.4) —	1536 (12.0) —	1536 Dead —	Dead —	Dead —
Coincident with sheep blood	10	12	3.5	....	....	24 (14.0)	0 (7.6)	24 (7.0)	768 (3.0)	768 (5.0) 800	6144 (4.8) 1000	6144 (6.2) 3200	6144 (4.0) 6400	3072 (8.0) 3200	1536 (10.2) 1600	1536 (14.5) 1600	768 (19.0) 3200	.... (10.4)	768 (6.8) 200	1536 (12.2) 200	1536 (10.8) 200	1536 (18.0) 0	1536 (17.6) 0
Coincident with sheep blood	5	13	3.3	....	....	0 (10.0)	48 (16.0)	0 (6.2)	3072 (7.0)	3072 (7.2) ?	3072 (9.8) 1000	6144 (9.0) 3200	3072 (10.5) 6400	768 (12.8) 3200	192 (9.4) 1600	192 (11.4) 800	192 (9.4) 400	.... (10.6)	192 (10.0) 400	192 (11.4) 200	389 (10.0) 200	168 (11.8) 0	168 (13.4) 0
Coincident with sheep blood	1	17	2.5	....	....	0 (9.0)	24 (26.0)	0 (4.2)	0 (16.0)	0 (7.6) 0	168 (6.6) 0	384 (10.6) 0	192 (7.4) 0	0 (5.0) 0	0 (5.8) 0	0 (4.0) 0	384 (4.7) 0	.... (6.2)	0 (10.9) 0	0 (7.4) 0	0 (9.4) 0	0 (8.6) 0	0 (12.0) —
5 days after injection of sheep blood	10	20	3.5	....	....	0	0	0	192 (12.0)	192 (8.0) 800	768 (4.0) 800	768 (4.9) 800	192 (7.6) 3200	96 (5.4) 1600	0 (21.0) 800	192 (9.5) 800	348 (10.0) 400	.... (13.6)	192 (7.6) 400	384 (7.5) 200	768 (13.8) 200	768 (15.2) 200	768 (16.0) 0
5 days after injection of sheep blood	5	23	3	....	....	0	0	96	96 (15.4)	96 (12.6) 0	1536 (17.0) 500 ?	768 (23.0) 0	384 (20.0) 0	0 (15.2) 0	0 (8.6) 0	0 (13.8) 0	384 (17.0) 0	.... (11.6)	0 (13.2) 0	384 (10.6) 0	0 (18.8) 0	(11.4) 0	(19.6) 0
5 days after injection of sheep blood	1	25	3	....	....	0	0	384	768 (14.0)	768 (10.6) 800	6144 (8.0) 3200	3072 (6.6) 6400	1536 (11.2) 6400	384 (9.4) 3200	384 (10.4) 1600	96 (9.6) 1600	0 (9.4) 3200	.... (5.4)	0 (11.2) 1600	48 (12.4) 1600	48 (10.2) 800	364 (11.0) —	364 (10.2) —
Controls	..	28	2.5	....	....	0	0 (8.2)	0 (7.4)	12288 (19.4)	12288 (12.0) 800	12288 (10.2) 1600	24000 (13.2) 3200	6144 (15.8) 6400	6144 (11.2) 6400	6144 (9.4) 3200	768 (12.4) 1600	3072 (14.6) 1600	.... (11.6)	1536 (17.0) 1600	1536 (16.0) 800	1536 (13.8) 400	1536 (15.8) 400	1536 (14.6) 400
		30	2.7	....	....	24	0 (21.6)	0 (26.4)	3072 (12.0)	6144 (10.8) 100+	12288 (12.4) 1600	6144 (17.9) 6400	1536 (27.4) 6400	768 (19.8) 6400	384 (19.4) 6400	24 (26.0) 3200	384 (24.0) 3200	.... (18.6)	1536 (28.8) 3200	1536 (27.0) 3200	1536 (20.4) 3200	1536 (22.0) 3200	1536 (18.0) 3200

Leukocyte counts (in parentheses) are given in fractions of thousands, i. e., 10.2 equals 10,200 leukocytes per cmm. of blood; the figures above the leukocyte counts give the lysin titers, while the figures below the leukocyte counts give the precipitin titers.

small hemorrhages. Microscopically, besides congestion the liver showed granular changes in the cells; the splenic sinuses were filled with pigment containing cells; the tubules of the kidneys contained blood in places and the epithelial cells were loosened; the heart muscles showed congestion and loss of striation, many nuclei being pycnotic; the marrow of the femur was congested, the marrow cells diminished in number.

Rabbit 6 (3.2 kilos).—Died 6 days after the intravenous injection of 39 millicuries. The axillary, inguinal and mesenteric nodes were hemorrhagic with loss of lymphoid cells; the liver pale, cells granular, nuclei hyperchromatic; the kidneys showed punctate hemorrhages with granular changes in the tubular cells; the spleen was small, there was yellow pigment in the trabeculae and many cells appeared to be undergoing disintegration; the myocardium contained small hemorrhages and there was loss of striation; in the lungs were hemorrhagic bronchopneumonic foci; the marrow of the femur was congested and the cells greatly diminished in number.

Rabbit 7 (3.2 kilos).—Died 6 days after the intravenous injection of 26 millicuries. The liver was friable, congested, nuclei large, protoplasm granular; the spleen was small and contained brownish yellow pigment; the kidneys were congested and there were detritus and casts in the tubules; the heart muscle showed diminished striation; the lungs extravasation of blood in the alveoli; the marrow of the femur and the lymphnodes loss of cells and there were hemorrhages in the marrow.

The remaining rabbits (8-13) in this series lived after receiving from 1 to 20 millicuries.

The most important changes appear to be congestion and small hemorrhages in the lungs, kidneys, and other organs with disintegrative changes and loss of cells in the spleen, marrow and lymphnodes. On the whole, the changes are not unlike those found by Bagg in the white rat and dog after the injection of active deposit.

THE EFFECT ON ANTIBODY FORMATION

In order to determine the effect of active deposit on antibody formation 30 rabbits, from 2.5 to 4 kilos in weight, were given intravenous injections of approximately 1, 5 and 10 millicuries, 4 days before, coincident with or 5 days after the intraperitoneal injection of about 10 c c of citrated sheep blood per kilo of weight, and the specific precipitin and lysin for sheep blood determined at regular intervals afterward. The technic for estimating the antibody content of the serum was the same as in the previous experiments of similar nature.[1] The results, which are illustrated in table 2, show that active deposit of radium emanation in doses of 1, 5 and 10 millicuries intravenously may cause a depression in the formation of the specific lysin which may be quite marked in individual rabbits. The effect seems more evident when the active deposit was injected 4 days before or coincident with the antigen rather than when injected 5 days after. Table 2 shows that

the active deposit of radium emanation in toxic nonlethal doses also has some depressant action on the formation of specific precipitin for sheep protein no matter whether given before, coincident with, or after the injection of the antigen. The action, however, is not regular. This seems to go hand in hand with the rapid disintegration chemically of the active deposit which has a rather indefinite lethal dose, being lethal at times within wide ranges for rabbits when given intravenously.

SUMMARY

Active deposit of radium is lethal to rabbits when given intravenously in amounts of approximately 8 to 10 millicuries per kilogram weight. In lethal amounts given intravenously the active deposit produces an initial leukocytosis with finally a marked diminution in the circulating leukocytes, mainly of the polymorphonuclears. This is associated with changes in the liver, lungs, lymph glands, spleen, suprarenals and kidneys frequently accompanied by capillary hemorrhages. These results are similar to those obtained with active deposit in white rats and dogs by Bagg. Given intravenously, active deposit in nonlethal amounts may have a depressing effect on the formation of lysin and to a less extent on precipitin for sheep blood.